

Microbial deterioration of cultural heritage and works of art — tilting at windmills?

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Abstract Microorganisms (bacteria, archaea and fungi), in addition to lichens and insect pests, cause problems in the conservation of cultural heritage because of their biodeteriorative potential. This holds true for all types of historic artefacts, and even for art made of modern materials, in public buildings, museums and private art collections. The variety of biodeterioration phenomena observed on materials of cultural heritage is determined by several factors, such as the chemical composition and nature of the material itself, the climate and exposure of the object, in addition to the manner and frequency of surface cleaning and housekeeping in museums. This study offers a review of a variety of well-known biodeterioration phenomena observed on different materials, such as stone and building materials, objects exhibited in museums and libraries, as well as human remains and burial-related materials. The decontamination of infected artefacts, exhibition rooms and depots incurs high expenditure for museums. Nevertheless, the question has to be raised: whether the process of biodeterioration of cultural heritage can or should be stopped under all circumstances, or whether we have to accept it as a natural and an implicit consecution of its creation. This study also highlights critically the pros and cons of biocide treatments and gives some prominent examples of successful and unsuccessful conservation treatments. Furthermore, an outlook on the future research needs and developments in this highly interesting field is given.

Keywords Biodeterioration phenomena · Microbial communities · Biocides · Conservation

Introduction

Biodeterioration can be defined as “any undesirable change in a material brought about by the vital activities of organisms” (Allsopp 2011). Bacteria, archaea, fungi and lichens as well as insect pests are constantly causing problems in the conservation of cultural heritage because of their biodeteriorative potential. This holds true for all types of historic artefacts and even for art made of modern materials (e.g., polymers; Sabev et al. 2006) in public museums and in private art collections. Fungi, bacteria and lichens are also found on mural paintings in churches, caves and catacombs, and even as biodeteriogens of architectural surfaces and stone monuments in outdoor environments (Ettenauer et al. 2010; Piñar and Sterflinger 2009; Saarela et al. 2004; Steiger et al. 2011; Sterflinger 2000; Urzi 2004). The oldest and most precious objects suffering from serious fungal invasions are rock art caves, such as the caves of Lascaux in France (Bastian and Alabouvette 2009).

Although the history of biodeterioration of houses and art is long and cases of red and green “leprosy” in houses have been described in the Bible (e.g., *Leviticus* Chap. 14, v. 36), its importance has been neglected for a long time, during which chemical and physical processes were believed to be the dominant factors of material decay. In recent decades, however, the dogma has changed and it is now generally agreed that fungi and bacteria not only cause serious aesthetical destruction of paintings, costumes, ceramics, mummies, books and manuscripts, they inhabit and penetrate into the materials, resulting in material loss, due to acid corrosion, enzymatic degradation and mechanical attack.

Decontamination of infected artefacts, exhibition rooms and depots results in high expenditure for museums (Allsopp et al. 2004; Cappitelli et al. 2009; Koestler et al. 2003; Mesquita et al. 2009; Nittérus 2000a; Pangallo et al. 2009; Sterflinger 2010). Allsopp (2011) stated that the annual world loss of non-food materials due to fungal attack is US\$40

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billion. However, the cultural and historical value of many paintings, books and manuscripts is inestimable and thus, cannot be expressed merely in terms of money. Nevertheless, the question has to be raised: whether the process of biodeterioration of cultural heritage can or should be stopped under any circumstances, or whether we have to accept it as a natural and implicit consecution of its creation.

Microorganisms associated with biodeterioration phenomena observed on materials of cultural heritage

The biodeterioration phenomena observed on materials of cultural heritage are determined by several factors: (1) the chemical composition and nature of the material itself, (2) the climate and exposure of the object, (3) the manner and frequency of surface cleaning and housekeeping in museums. Some well-known examples are detailed below.

Biodeterioration of stone and building materials

Microorganisms contribute significantly to the overall deterioration phenomena observed on stone and other building materials, such as concrete, mortar, slurries and paint coatings, glass and metals used in architecture (Piñar and Sterflinger 2009). On building stone exposed to the environment, fungi may be the most important biodeteriorative organisms because they are extremely erosive (Scheerer et al. 2009; Sterflinger 2000). Depending on the physical properties of the material, fungi may penetrate inside the stone. The phenomenon of bio-pitting — the formation of pits in sizes ranging up to 2 cm in diameter and depth in stone — is caused mainly by black fungi. Bio-pitting occurs predominantly on marble and limestone, but it has also been observed on antique glass (Piñar et al. 2013a). There are two major morphological and ecological groups of stone-inhabiting and stone-dwelling fungi. These have adapted to different environmental conditions. In moderate or humid climates, the fungal communities on rock are dominated by hyphomycetes (mold) that form mycelia (hyphal networks) in the porous space of the stones (Sterflinger 2000; Rosling et al. 2009). Since the settlement of spores from the air is the first step for fungal colonization, the species diversity of stone fungi is rather similar to the diversity of common airborne spores. *Alternaria*, *Cladosporium*, *Epicoccum*, *Aureobasidium* and *Phoma* are the most important species (Sterflinger and Prillinger 2001). In arid and semi-arid environments, such as those found in the Mediterranean area, the climatic conditions are too extreme for most hyphomycetes, therefore the communities shift towards the so-called black yeasts and microcolonial fungi. Black fungi belonging to the genera *Hortaea*, *Sarcinomyces*, *Coniosporium*, *Capnobotryella*, *Exophiala*, *Knufia* and *Trimmatostroma* form small black

colonies on and inside the stone and often occur in close association with lichens (Sterflinger 2005). Due to the thick walls they develop, fungi also resist chemical attack and, therefore, resist biocides and other anti-microbial treatments. Black fungi dwell deep inside granite, calcareous limestone and marble, which they erode by both chemical and mechanical attack. They are the main culprits for the phenomenon of bio-pitting. Due to the strong melanization of the cell walls, stones colonized by these fungi exhibit black spots or may be completely covered by a black layer. In addition to outdoor environments, black fungi are also found on rock surfaces of caves and catacombs (Saarela et al. 2004) especially where the naturally high humidity has been actively decreased in order to suppress algal growth on precious wall paintings.

Cyanobacteria, algae and lichens contribute to the weathering of stone in humid as well as in semi arid and arid environments (Cutler et al. 2013; Lamprinou et al. 2013). They produce a characteristic phenomenon consisting of large green-black stains (Figs. 1 and 2a). The ability of cyanobacteria to adapt to different light qualities by chromatic adaptation, also allows them to develop on stone in archaeological hypogea with low light intensities, as in the case of crypts, caves and catacombs. There, they may be one of the most important deterioration agents for wall paintings and inscriptions. In such subsurface environments *Eucapsis*, *Leptolyngbya*, *Scytonema* and *Fischerella* have been the most frequently encountered cyanobacterial taxa (Bellezza et al. 2003).

The role of chemoheterotrophic bacteria in the weathering of rock probably depends largely on the environmental conditions: while bacteria might evolve in humid environments and form biofilms within the porous space of building stone, in arid and semi arid environments their occurrence might be limited. However, chemoheterotrophs are not only contributing to the weathering of rock. This group of microorganisms has been shown to have some impact on the consolidation of rock and plaster because they enhance calcium carbonate precipitation by passive and active processes. Strains of *Bacillus cereus* and *Myxococcus xanthus* have been used to

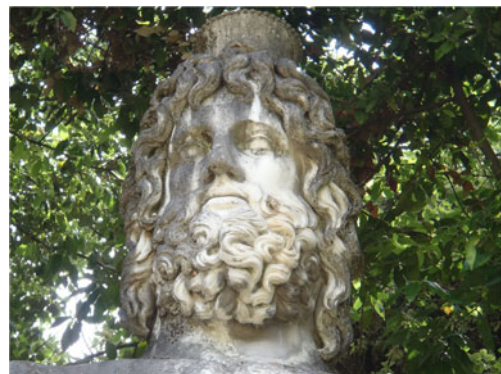


Fig. 1 Sculpture made of white Carrara marble with black discolorations caused by fungi and lichens; Boboli Park, Florence, Italy (Photo: Sterflinger)

Fig. 2 **a** Green algal and cyanobacterial stains on mortar surfaces in the castle of Rappottenstein (Austria). **b** Rosy stains characteristic for halophilic and halotolerant archaea and bacteria. (Photos: Ettenauer)



actively bio-induce calcite precipitation to reinforce monumental stone (Castanier et al. 1999; Ettenauer et al. 2011; Fernandes 2006; Jimenez-Lopez et al. 2007; Piñar et al. 2010; Rodriguez-Navarro et al. 2003; Tiano et al. 1999).

Members of the *Actinobacteria* phylum inhabit stone more effectively than most of the single-celled bacteria. This fact can be attributed to their filamentous growth and also to their effective utilization of various nitrogen and carbon sources (Saarela et al. 2004). Heterotrophic bacteria include a variety of genera such as *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Paenibacillus*, *Flavobacterium*, *Pseudomonas*, *Micrococcus*, *Staphylococcus*, *Nocardia*, *Mycobacterium*, *Streptomyces* and *Sarcina*, which are the species most frequently isolated from wall paintings (Bassi et al. 1986; Ciferri 1999; Heyrman et al. 1999; Palla et al. 2002; Pangallo et al. 2012; Suihko et al. 2007) but also in caves and catacombs (De Leo et al. 2012). In some cases, especially when organic layers — e.g., saccharose, starch or cellulose — have been applied for the fixation of a wall painting, common indoor fungi like *Cladosporium* or *Alternaria* may also inhabit wall paintings and plaster (Fig. 3a, b).

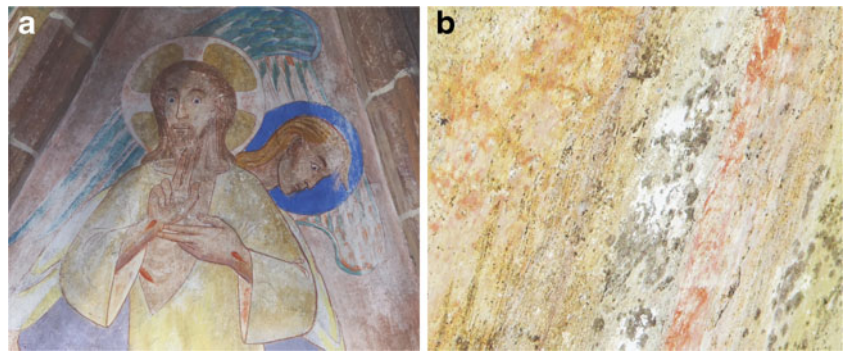
A well-known phenomenon often observed on buildings and wall paintings, especially on those under non-controlled climatic conditions, is the formation of salt efflorescence on the wall surfaces (Amoroso and Fassina 1983). Salt may be available in the wall itself, from biological processes (ammonium salts) or simply due to co-migration with infiltrating water. Due to changes in physical parameters, i.e., temperature or humidity, salts can precipitate on the exposed surfaces. The crystallization of salts on walls and wall paintings results in a destructive effect. Some salts can crystallize to different hydrates, occupying a larger space and producing an additional pressure that eventually leads to material loss and destruction due to cracking and detachment of the walls (Saiz-Jimenez and Laiz 2000; Piñar et al. 2009, 2013b). Moreover, the salt efflorescence mimics the conditions found in extreme habitats favoring the proliferation of halotolerant/halophilic microorganisms.

Halophilic species of the *Gammaproteobacteria* (such as the genera *Idiomarina*, *Salinisphaera* and *Halomonas*) and *Firmicutes* (*Halobacillus* and *Bacillus* spp.), but also species of the phyla *Bacteroidetes* and *Actinobacteria* (as *Rubrobacter*) have often been detected on salt-attacked monuments. In addition, the most important genera of archaea found in such environments are *Halococcus* and *Halobacterium* (Ettenauer et al. 2010, 2013 submitted; Imperi et al. 2007; Jurado et al. 2012; Laiz et al. 2009; Piñar et al. 2001, 2009, 2013b; Saiz-Jimenez and Laiz 2000). Many of these microorganisms contain carotenoid pigments such as β -carotene, α -bacterioruberin and derivatives, and salinixanthin in their cell membranes (Oren 2009). Their proliferation produces typical rosy stains on the wall surfaces, significantly influencing the optical appearance of wall paintings and historical plaster (Fig. 2a, b).

Biodeterioration in museums and libraries

In museums and collections, as well as in libraries, fungi play the most important role in biodeterioration. Infections are mostly airborne — with significant seasonal variations — and high numbers of spores can accumulate in dust layers (Kaarakainen et al. 2009). Poor ventilation and non-homogeneous surface temperature can produce water condensation points and local micro-climates with higher water availability than in the rest of an indoor environment. These circumstances are favourable to some fungal species; as a result, these are able to proliferate in places where the overall environmental conditions would otherwise appear to be hostile to microbial life. Typical fungal infections in libraries, colonizing documents made of paper, are caused by species of slow-growing *Ascomycetes* as well as mitosporic xerophilic fungi (fungi that thrive in materials with a low water activity, i.e., $a_w=0.70-0.85$) of the genera *Aspergillus*, *Paecilomyces*, *Chrysosporium*, *Penicillium* and *Cladosporium* (Pinzari and Montanari 2011). Nevertheless, it is worth noting on special cases of mono-specific infections inside compact shelving,

Fig. 3 **a, b** Growth of the fungus *Cladosporium* sp. on a modern wall painting (Karl Weiser 1952), Weyregg, Austria. (Photos: Sterflinger)



which have been attributed mainly to fungal species belonging to the *Eurotium* genera, such as *Eurotium halophilicum* (Montanari et al. 2012).

A well-known phenomenon that some authors attribute to fungal activity on paper is the so-called “foxing”, consisting of small and isolated rusty red-brownish spots which are often not directly linked to structural degradation of the substratum (Gallo and Pasquariello 1989). Since the earliest studies, foxed spots have been controversially attributed to biological agents (fungi and bacteria) or to chemical factors (iron oxidation, organic and inorganic dust particles, etc.). Recent studies on the foxing problem, both via scanning electron microscopy, and by chemical and microbiological analysis, also led to inconclusive results (Arai 2000; Choi 2007), but recent research has agreed on the fungal nature of the phenomenon (Michaelsen et al. 2009, 2010; Rakotonirainy et al. 2007) and on the implication of bacteria in the deterioration of paper (De Paolis and Lippi 2008; Michaelsen et al. 2010).

A very different infection can occur in libraries and archives when water suddenly becomes available, such as in the case of flooding. In this case, molds associated with water damage consist of fungal species that need a high water activity. These molds can produce coloured stains (i.e., *Chaetomium* spp., *Monoascus* spp., and *Epicoccum* spp.), strong odours (i.e., *Trichoderma* spp.) and toxic compounds (i.e., *Stachybotrys* spp.).

Fungal degradation of library materials and paintings causes different kinds of damage depending on the species

of organism responsible for the attack and the characteristics of the substratum. Damage can occur because of mechanical stress, production of staining compounds or enzymatic action (Blyskal 2009; López-Miras et al. 2013; Pinzari et al. 2010; Santos et al. 2009; Sterflinger 2010). Most of the filamentous fungi associated with the damage of paper and oil paintings on canvas can dissolve cellulose fibres with the action of cellulolytic enzymes, or may discolour the support, dissolve glues and inks or degrade the oil binders (Fig. 4a).

The degradation of documents made of parchment — which is mainly composed of collagen — is a complex process, which involves the chemical oxidative deterioration of amino acid chains and hydrolytic cleavage of the peptide structure. Microorganisms can hydrolyze collagen fibres and other protein-based materials, but can also modify the inorganic components, or produce pigments and organic acids which discolour the parchment. Bacteria displaying proteolytic activities play a major role in the deterioration of ancient documents and books made of parchment. Species belonging to the genera *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Virgibacillus* and *Micromonospora* have been isolated from deteriorated parchments (Kraková et al. 2012). In addition, some alkaliphilic bacteria (microbes that thrive in environments with a pH of 9 to 11) and several species of the *Actinobacteria* have been detected in connexion with a typical damage phenomenon, namely a parchment discoloration consisting of purple spots (Pinzari et al. 2012; Piñar et al. 2011; Strzelczyk and Karbowska-Berent 2000). Parchment

Fig. 4 **a** White fungus growing in the craquelées of an oil painting (Stift Lilienfeld, Austria); **b** growth of black fungi on the wall paintings of the Paulus grotto near Ephesus, Turkey. (Photos: Sterflinger)



also provides good conditions for the development of proteolytic fungi, among which numerous representatives of *Ascomycetes* such as *Chaetomium* and *Gymnoascus*, as well as mitosporic fungi in the genera *Acremonium*, *Aspergillus*, *Aureobasidium*, *Epicoccum*, *Trichoderma*, and *Verticillium*.

Biodeterioration of human remains and related buried or exhibited materials

Very special cases of biodeterioration occur whenever nutrient-rich materials are involved and the climate is non-controlled. This is the case for mummies and related materials, such as clothes, documents or stuffing materials buried or exhibited; conserved in churches and crypts (Jurado et al. 2010; Pangallo et al. 2013; Piombino-Mascali et al. 2011; Piñar et al. 2013b). A very impressive example of this kind of deterioration is represented by the mummies of the Capuchin Catacombs in Palermo, Italy. First observations revealed a heavy mold contamination on the surface of the mummies, but deep molecular analyses revealed complex microbial communities, consisting of bacteria, archaea, and fungi, colonizing the mummies and related materials. Sequences related to specialized microorganisms belonging to taxa well known for their cellulolytic and proteolytic activities were detected on cellulosic and keratin- and collagen-rich materials, respectively. Additionally, sequences related to the human skin microbiome and to some pathogenic bacteria (order Clostridiales) and fungi (genus *Phialosimplex*) were identified on the mummies. There are also other well-known examples which show the colonization of preserved bodies by opportunistic fungi, such as the case of the restoration of the body of Ramses II, performed in Paris in 1976–1977 (Mouchacca 1985) and the high fungal contamination of the air and dust of the Egyptian mummy chamber at the Baroda Museum in India (Arya et al. 2001). Additionally, saprophytic fungi and bacteria were isolated from a mummy from the collection of the Archaeological Museum in Zagreb, Croatia (Čavka et al. 2010). All these studies clearly demonstrate that specialized microorganisms are threatening the conservation of human remains and related materials, and that high concentrations of air-borne fungal spores may even pose a potential health risk for visitors (Piñar et al. 2013b).

To kill or not to kill? Antimicrobial treatments in restoration and conservation

For disinfection of recent and progressive microbiological damage, a limited range of physical and chemical methods are available (Allsopp et al. 2004). Chemical treatments include liquid biocides and fumigation with gases. The choice of an appropriate biocide is limited by the European Union's Biocidal Products Directive (BPD) (<http://ec.europa.eu/environment/biocides/index.htm>). Although the number of

chemical classes listed by Paulus (2004) includes a wide variety, such as alcohols, aldehydes, phenols, acids, acid esters, amides, carbamates, dibenzamidines, pyridines, azoles, heterocyclics, activated halogen compounds, surface active agents, organometallics and oxidizing agents, the number of products suitable for cultural heritage is comparatively limited because only a small number of agents have been tested with respect to their compatibility with historic materials, such as pigments, organic binders or paper, and only a very few studies exist on the long term effects of the biocides, such as possible colour changes or degradation products. Biocides frequently used in restoration are: (1) formaldehyde releasers (Sterflinger and Sert 2006; Pinar et al. 2009), (2) quaternary ammonium compounds with an optimal chain length of C14–C16 (Diaz-Herrera et al. 2013), (3) isothiazolinone, a more recent biocide, which was documented to be not only effective but even preventive on paper objects (Polo et al. 2010) and 4) the most common disinfectant used in microbiology: ethanol can also have a good fungitoxic effect if the contact time is at least 2–3 min (Nittérus 2000b). A broad spectrum of chemical and non-chemical mass treatments has been utilized to kill microfungi attacking paper objects in an attempt to inhibit degradation (Magaudha 2004). Ethylene oxide (EtO) fumigation is banned in some countries because it is extremely toxic, but it still represents the most efficacious system for mass treatment of mouldy library materials. Gamma radiation is very effective against fungi and their spores. Since the dose for fungi must be in excess of 10–20 kGy (Nittérus 2000a), this method also affects many materials and its application is restricted. The application of gamma rays can result in cumulative depolymerisation of the underlying cellulose and in severe ageing characteristics (Adamo et al. 1998; Butterfield 1987).

Besides the compatibility with the materials of the treated artefacts, the most challenging aspect of biocide treatments is the fact that, in many cases, objects are infested by a mixed community of microorganisms with different levels of susceptibility towards the chemical compound applied. For microbiologists it is quite easy to understand that a biocide treatment might therefore exert a selective pressure on the microbial community and, in the worst case, the community may be turned into one that is less sensitive or even resistant to the biocides, and might become even more harmful to the object. Prominent and notorious examples are the so-called Cave of St. Paul in Ephesus (Turkey) and the wall paintings of Lascaux (France). In the Cave of St. Paul, a massive algal and cyanobacterial bloom covered the early Christian wall paintings. After several treatments with quaternary ammonium compounds, a more resistant community — which included melanized fungi — developed, causing severe aesthetical damage to the surfaces (Pillinger et al. 2008) (Fig. 4b). In the Lascaux Caves, a spectacular series of biocide treatments were carried out, starting in 1963, with the last being reported in 2009 (Martin-Sanchez et al. 2012). Here, antibiotics, such as penicillin, streptomycin and kanamycin —

but also formol (10 % aqueous solution of formaldehyde), various products based on benzalkonium chloride and isothiazolinone — were applied. These successive treatments triggered the development of white fungal stains caused by *Fusarium solani*, the growth of resistant *Pseudomonas fluorescens* strains and finally, the growth of melanized fungal species, such as *Ochroconis lascauxensis*, *O. anomala* and *Exophiala castellanii* (Saiz-Jimenez et al. 2012).

In contrast to this, good results were achieved against a mono-specific infestation of *Aspergillus glaucus* inhabiting the painting and fixation layer of the 12th century wooden ceiling in Zillis (Switzerland). There, the individual wooden panels of the ceiling were successfully treated with the application of organotin (TBTO), a biocide that is efficient but which has been abandoned in Europe because of its high environmental toxicity. However, also in Zillis, the most important control factor was a system for climate control (Bläuer-Böhm et al. 1997; Böhm et al. 2001).

In the past — especially in the 1960s and 1970s — a number of highly toxic organochloride compounds like lindane or pentachlorophenole (PCB) were used for decontamination of wooden objects and textiles. Since these agents are chemically very stable, they might still persist in many of the objects treated and thus are a health risk for restorers that handle these objects today. Other past treatments might hamper or falsify biological, chemical or physical analysis. Fumigation with ethylenoxide, for example, interferes with biological analysis since it intercalates with DNA and RNA which cannot be recovered anymore (Michaelsen et al. 2013). The lack of documentation in the past complicates today's restoration and conservation work. Today, documentation of objects and their restoration history is one of the most important responsibilities in conservation as a basis for our progeny.

Treatments and monitoring

One of the major obstacles in treating contaminated art works with biocides and physical methods like Gamma radiation or heat was, and still is, the lack of appropriate monitoring methods. For the taxon analysis of microbial communities on art works, it is widely accepted that not all fungi, and only an extremely small fraction of archaea and bacteria, can be cultivated on laboratory media and that molecular methods based on DNA are necessary to evaluate the microbial diversity in a sample (Ettenauer et al. 2012; González and Saiz-Jimenez 2005; Laiz et al. 2003; Michaelsen et al. 2006; Piñar et al. 2001; Schabereiter-Gurtner et al. 2001). Curiously, viable cell counts are still the method of choice to prove microbial activity versus non-activity, if any test is carried out to monitor the effect of an antimicrobial treatment at all. Since the late 1980s, when it was generally agreed that microorganisms played a considerable role in the preservation of art objects and historical

buildings, significant effort was applied to ascertaining the biodiversity in the component materials of works of art. This was an important basis for innovative and optimized preservation concepts. Today, it is absolutely necessary to complement these data by studying the physiological activity of the various microbes on and in materials (a) in order to get a deeper understanding of biodeterioration processes, (b) to be able to monitor the effect and success of antimicrobial treatments and (c) to develop alternative and non-toxic treatment methods, e.g., special climatization concepts in order to stop or to slow down the biodeteriorative action of the microorganisms. In the past, several attempts were made to quantify microbial activity based on chemical reactions: Sterflinger et al. (1994) developed a non destructive method, the “respiration bell-jar” to trap CO₂ in order to monitor respiration on stone surfaces. Redox indicators such as triphenyltetrazoliumchloride were used to confirm and evaluate microbial activity on decaying stones (Warscheid 1990). Recently, many companies have offered luminometers that detect and quantify ATP in swab samples and give an estimation of biological activity on surfaces like paper, paintings or other materials (Berthold and Tarkkanen 2013; Rakotonirainy and Arnold 2008). While these methods give a rough estimation of the microbial activity in general, analysing the expression of genes would give detailed information about the metabolic state and about the biodeterioration process and potential — as in, for example, following the activity of cellulolytic and keratinolytic enzymes on paper and parchment (Kráková et al. 2012). Although RT qPCR is a routine tool for scientific questions nowadays, it is still not used for routine monitoring of treatments, and studies on RNA in samples of cultural heritage are still rare (Martin Sanchez et al. 2013; Michaelsen et al. 2013; Portillo et al. 2008, 2009). This is because the costs for molecular analysis are still high in relation to the overall costs that are usually available for the restoration and conservation of an object. However, recent genomics and transcriptomics technology opens more possibilities to understanding the activity and function of whole microbial communities. Sequencing of meta-transcriptomes and metagenomes, with the aid of next-generation sequencing technology, could assist in understanding how historic materials are attacked by microbes, how microbes interact with those materials and with each other (e.g., in a biofilm), and in monitoring specifically the effect of biocide treatments on the viability, the function and possible community shifts. This would also help to overcome the so-called “viable but not cultivable” state in bacteria that can occur as a reaction to antibiotic and biocide treatments (Oliver 2009).

Outlook

The most important factors for prevention of biogenic damage on historic objects are: (1) climate control, (2) frequent cleaning

and (3) phenomenological monitoring (Barton and Wellheiser 1985; Dicus 2000; Pinzari 2011; Sterflinger 2010). The importance of simple cleaning is still underestimated, despite the fact that it is well known that dust layers on objects carry high numbers of fungal spores and bacteria, and also serve as a nutrient source for those organisms. Microbiologists must increase the awareness of these preventative measures by consulting with and instructing restorers, preservationists and museum curators. We must learn from the mistakes made with biocide treatments in the past, and apply the following principles:

- (1) More emphasis must be focused on simple prevention measures such as the cleaning of dust layers and frequent observation of objects.
- (2) Biocide treatments must be applied with extreme caution and only after a stringent series of tests adapted to the requirements of a particular object. In restoration and conservation, exceptional rules are necessary for the application of efficient toxic substances, which may be not listed in the EU biocide directive.
- (3) More effort is necessary in the development of alternative decontamination methods, e.g., the gamma radiation (Magaudda 2004) modification of light (Albertano et al. 2005) and micro-climates (Camuffo 1998; Pinzari and Montanari 2011).
- (4) Monitoring methods must be optimized in order to be able to assess the effects of conservation treatments, climate change or biocide application. This could be done based on state of the art microbiological methods such as genome and transcriptome sequencing.
- (5) In the case where we cannot ensure that a freshly excavated object can be preserved and protected against biodeterioration, it should remain buried in soil or under layers of paint or plaster (e.g., for wall paintings) until better methods are available for preservation. A paradigm change is necessary in order to learn that not everything that is discovered must (or can) be exhibited and opened to the public.

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